MAJOR ARTICLE

Human T Lymphotropic Virus Types I and II Western Blot Seroindeterminate Status and Its Association with Exposure to Prototype HTLV-I

Karen Yao,[1,4] Michie Hisada,[2] Elizabeth Maloney,[2,a] Yoshihisa Yamano,[1,a] Barrie Hanchard,[5] Rainford Wilks,[5] Maria Rios,[3] and Steven Jacobson[1]

[1]Viral Immunology Section, Neuroimmunology Branch, National Institute of Neurologic Disorders and Stroke, National Institutes of Health, and [2]Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, [3]Food and Drug Administration, Rockville, and [4]Department of Biology, Johns Hopkins University, Baltimore, Maryland; [5]University of the West Indies, Mona Kingston, Jamaica

Human T lymphotropic virus types I and II (HTLV-I/II) Western blot (WB) seroindeterminate status, which is defined as an incomplete banding pattern of HTLV protein Gag (p19 or p24) or Env (GD21 or rgp46), is commonly observed. To investigate the significance of this finding, we examined HTLV-I/II serostatus and HTLV-I proviral load in 2 groups of individuals with WB seroindeterminate status. Low proviral loads were detected in 42% of patients with neurologic symptoms and 44% of voluntary blood donors. These data suggest that a subset of WB seroindeterminate individuals may be infected with prototype HTLV-I. To confirm this hypothesis, we evaluated HTLV-I/II serostatus and proviral load in prospectively collected specimens from 66 WB seronegative patients who had received HTLV-I–infected blood products by transfusion. Eight individuals developed WB seroindeterminate profiles after the transfusion. In addition, using a human leukocyte antigen type A*201–restricted HTLV-I Tax11-19 tetramer, we detected virus-specific $CD8^+$ T cells in peripheral blood mononuclear cells from WB seroindeterminate patients. These $CD8^+$ T cells were effective at targeting HTLV-I–infected cells. Collectively, the results suggest that HTLV-I/II WB seroindeterminate status may reflect a history of HTLV-I exposure. Our findings warrant further investigation of the possible clinical outcomes associated with WB seroindeterminate status.

Human T lymphotropic virus type I (HTLV-I) is a retrovirus that is endemic in southern Japan, the Caribbean basin, and parts of West Africa, Melanesia, South America, and the Middle East [1]. It is the etiological agent of adult T cell leukemia (ATL)—an aggressive malignancy of $CD4^+CD25^+$ T lymphocytes—and HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP)—a chronic progressive inflammatory neurodegenerative disorder [2]. Transmission, which requires cell-to-cell contact, occurs primarily through breast-feeding from an infected mother, exposure to HTLV-I–contaminated blood products, or sexual contact with an infected person [1]. Perinatal infection is thought to be associated with a heightened risk of developing ATL after a long latency period [3], whereas acquiring infection later in life may lead to the onset of HAM/TSP shortly after infection [1, 4].

Infection with HTLV-II, a distinct retrovirus that is closely related to HTLV-I, is found primarily in some American Indian tribes and injection drug users [5–7]. HTLV-II shares a number of important biological properties with HTLV-I, such as similar routes of transmission, and it has also been shown to be associated

Received 5 April 2005; accepted 6 July 2005; electronically published 28 December 2005.

Present affiliations: Division of Viral and Rickettsial Diseases, Centers for Disease Prevention and Control, Atlanta, Georgia (E.M.); Third Department of Medicine, Kagoshima University School of Medicine, Kagoshima, Japan (Y.Y.).

Potential conflicts of interest: none reported.

Financial support: National Institute of Neurologic Disorders and Stroke, National Institutes of Health.

[a] K.Y. and M.H. contributed equally to the work.

Reprints or correspondence: Dr. Steve Jacobson, Neuroimmunology Branch, National Institute of Neurologic Disorders and Stroke, National Institutes of Health, 9000 Rockville Pike, Bldg. 10, Ste. 5N214, Bethesda, MD 20892 (jacobsons@ninds.nih.gov).

The Journal of Infectious Diseases 2006;193:427–37

0022-1899/2006/19303-0013$15.00

with a HAM/TSP-like neurological disorder [8, 9]. However, the association of HTLV-II infection with neurological disorders is much weaker than that observed between HTLV-I and HAM/TSP. Moreover, HTLV-II infection has not been consistently associated with any malignancy. It has been estimated that ~15–20 million people worldwide are infected with HTLV-I [1]. In the United States alone, an estimated 50,000 people are infected with HTLV-I, and ~200,000 people are infected with HTLV-II [9]. Mandatory screening of blood supplies from voluntary donors implemented in the mid-1980s in countries such as Japan, Canada, and the United States has significantly reduced the risk of HTLV-I/II transmission through blood transfusion. However, in many western European and South American countries, screening procedures were not put into place until the 1990s. More importantly, in Africa, which is considered to be the largest reservoir of HTLV-I, such screenings are still not performed because of the expenses associated with HTLV-I testing.

ELISA and the particle agglutination assay are 2 assays that are commonly used to screen for serum antibodies against HTLV-I or -II. Infection, however, is confirmed by the detection of antibody against viral structural proteins by Western blot (WB) analysis. The incorporation of recombinant type-specific glycoproteins rgp46-I or rgp46-II on the WB assay enables the distinction of antibody reactivity to HTLV-I from that to HTLV-II. It has been shown that some individuals with positive ELISA results demonstrate incomplete antibody reactivity to HTLV-I or -II antigens; these individuals are considered to have HTLV-I/II WB seroindeterminate status [10–13]. The reported prevalence of WB seroindeterminate status ranges from as high as 67% in patients from Zaire with neurologic diseases [13] to 4.1% in HIV-1–positive patients from Brazil [14] to variably low in asymptomatic individuals—0.02%, 0.5%, 0.6%, and 0.1% in blood donors from Taiwan, French West Indies, Brazil, and Argentina, respectively [15–18]. More importantly, we previously demonstrated that ELISA-negative individuals could have WB seroindeterminate status [12], which suggests that the prevalence of HTLV-I/II WB seroindeterminate status may be higher than what has been estimated.

However, to date, the clinical significance of HTLV-I/II WB seroindeterminate status has remained elusive. The goal of the present study was to investigate the association of an HTLV-I/II WB seroindeterminate status with prior exposure to HTLV-I. Several hypotheses have been proposed for the serologic finding of HTLV-I/II WB seroindeterminate status. Waziri et al. [19] found a full-length 9-kB sequence of prototype HTLV-I in a B cell line derived from an individual with an HTLV-I/II WB seroindeterminate status, which suggests the possibility that WB seroindeterminate status is the result of low-level prototype HTLV-I infection. Cross-reactivity to other infectious agents—for example, *Plasmodium falciparum*—has been proposed as an explanation for HTLV-I/II seroindeterminate WB results in areas where malaria is endemic [20]. The possibilities of infection with a novel retrovirus or defective infectious agent have also been considered [21–23].

SUBJECTS, MATERIALS, AND METHODS

Study subjects. Three different cohorts of individuals with HTLV-I/II WB indeterminate status were evaluated. (1) Twelve individuals with WB seroindeterminate status were identified among consecutively referred patients at the neurologic clinic of the National Institute of Neurologic Disorders and Stroke (NINDS). (2) Nine WB seroindeterminate blood donors were identified at the New York Blood Center. (3) Sixty-six Jamaicans who received a transfusion of HTLV-I–contaminated blood products between 1987 and 1988, before the implementation of HTLV-I screening tests, had been prospectively monitored by the University of the West Indies (UWI) and the National Cancer Institute (NCI). These patients were seen in the clinic monthly after the transfusion for up to 3 months and every 3 months thereafter [24]. Informed consent was obtained from all participants. The institutional review boards of NINDS, UWI, and NCI approved the study protocol.

HTLV-I serologic analysis. Serum and plasma samples were tested for HTLV-I/II antibodies by ELISA (Abbott Laboratories), in accordance with the manufacturer's instructions. Reactive samples were confirmed with a WB assay (HTLV Blot 2.4; Genelabs Technologies) at a 1:100 dilution. Indeterminate samples were repeated at a 1:50 dilution. Band strengths were read against an 11-score gray scale. Detection of both of the Gag proteins (p19 and p24) and 1 of 2 Env proteins (gp21 and rgp46) defined HTLV-I seropositivity.

DNA extraction. Genomic DNA was extracted from 2×10^6 peripheral blood mononuclear cells (PBMCs), using the Puregene Genomic DNA Purification Kit (Gentra Systems), in accordance with the manufacturer's specifications.

Detection of HTLV-I provirus in PBMCs by polymerase chain reaction (PCR)/liquid hybridization. DNA from 9 healthy blood donors was isolated from PBMCs using the protocol described above. High-DNA throughput PCR (HTPCR) followed by liquid hybridization with a specific internal probe, labeled with P^{32} for both the *tax* and *pol* gene regions, was performed as described elsewhere [25, 26]. The primers and probes used were SK43/44/45 and SK110/111/112, for *tax* and *pol,* respectively.

Quantitation of HTLV-I proviral load. The HTLV-I proviral load in PBMCs was measured by real-time TaqMan PCR using the ABI PRISM 7700 Sequence Detector (Perkin Elmer/Applied Biosystems) as described elsewhere [27]. The amount of HTLV-I proviral DNA was calculated as [copies of HTLV-I (pX)/(copies of β-actin/2)] $\times 10^6$ cells. Samples were tested in triplicate. Results were considered to be positive if all 3 values showed detectable levels.

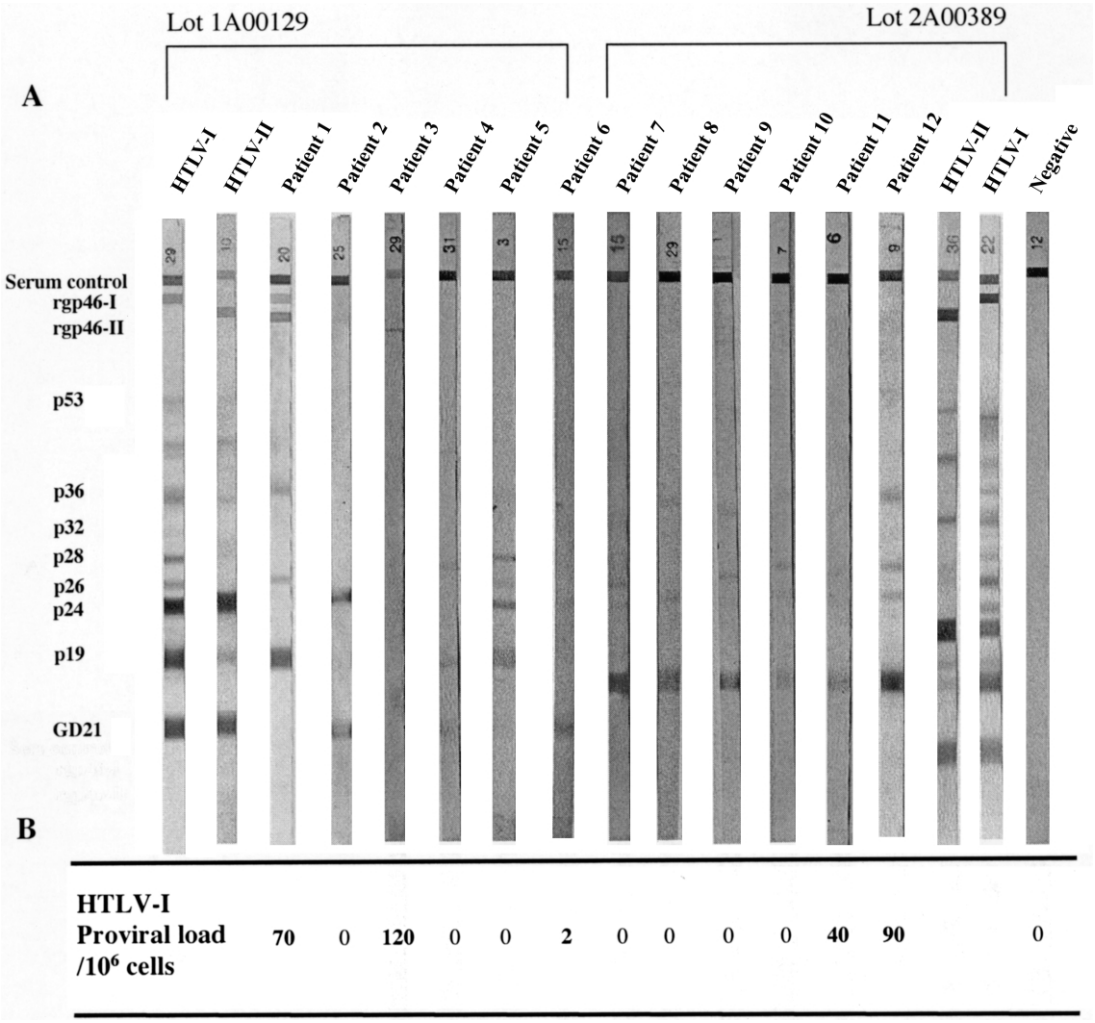

Figure 1. Human T lymphotropic virus type I and II (HTLV-I/II) seroindeterminate Western blot (WB) and real-time TaqMan quantitative polymerase chain reaction (PCR) results for the HTLV-I *pX* gene of peripheral blood mononuclear cells (PBMCs) from patients with neurologic symptoms at the National Institute of Neurologic Disorders and Stroke. *A,* Results of HTLV-I/II WB of 12 patients with neurologic symptoms. Patients 7–12 had a HTLV-I/II Gag seroindeterminate status, classified by the lack of antibody reactivity to any Env proteins and p24. Patient 1 had antibody reactivity against both rgp46-I and rgp46-II in addition to p36, p26, and p19. Patient 2 had antibody reactivity to both p24 and GD21. Patient 3 had an uncharacterized band below rgp46-II. Patient 4 had banding patterns for p26, p19, and GD21. Patient 5 lacked antibody to Env proteins but had antibody to p19, p24, and p28. Patient 6 had anti-GD21 antibody reactivity. HTLV-I–and HTLV-II seropositive and seronegative WBs are shown as controls. The brackets indicate lot nos. of WBs used. *B,* Real-time TaqMan quantitative PCR measurements of HTLV-I proviral load per 10^6 cells in the PBMCs below the corresponding WB. Positive PCR values are shown in bold type.

Tax11-19 tetramer staining and sorting. HTLV-I Tax11-19 tetramer staining was performed as described elsewhere [28]. PBMCs were first isolated with anti–human CD8 magnetic beads (Miltenyi). fluorescence-activated cell sorting was performed by gating on HTLV-I Tax11-19 tetramer–positive cells. T cell lines were generated as described elsewhere [29].

Cytotoxic T lymphocyte (CTL) assay. HTLV-I–specific CTL activity was evaluated as described elsewhere [1]. Briefly, the HmyA2.1 B cell line was pulsed with 5 μmol/L HTLV-I Tax11-19 and HIV Gag (negative control) peptides for 30 min at 37°C. HTLV-I–infected $CD8^+$ CTLs derived from a patient with HAM/TSP were used as a positive control. After incubation, HmyA2.1 cells were washed and plated at a concentration of 3.5×10^3 cells/well with T cell lines or HAM/TSP CTLs at different effector:target ratios for 4 h at 37°C. The percentage of specific lysis was calculated as (experimental release − spontaneous release)/(maximum release − spontaneous release) × 100 (%).

RESULTS

HTLV-I provirus in PBMCs from patients with neurologic symptoms. Patients with diverse neurologic complaints were referred to the NINDS to be evaluated for neurologic diseases. Antibody reactivity to HTLV-I/II was assessed by ELISA for all

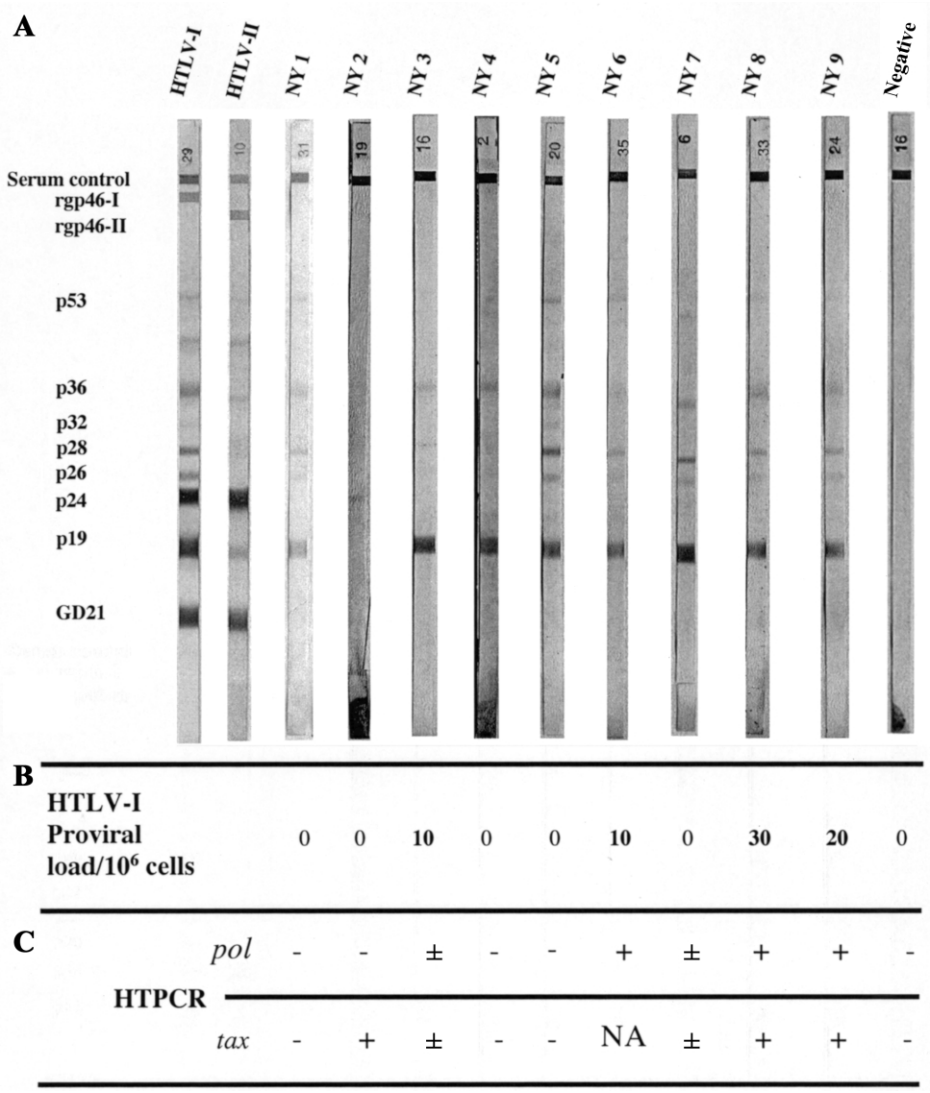

Figure 2. Human T lymphotropic virus type I and II (HTLV-I/II) seroindeterminate Western blot (WB) and real-time TaqMan quantitative polymerase chain reaction (PCR) results for peripheral blood mononuclear cells and high-DNA throughput PCR (HTPCR)/liquid hybridization results from healthy blood donors. *A,* HTLV-I/II seroindeterminate WB of ELISA-positive serum. *B,* HTLV-I proviral load per 10^6 cells, measured by real-time TaqMan quantitative PCR. *C,* HTPCR and detection by liquid hybridization with P^{32}-labeled specific internal probe. *pol* and *tax* are regions encoding the HTLV-I reverse transcriptase and regulatory proteins, respectively. + denotes detection of amplification product, − denotes no amplification, and ± denotes 2 rounds of amplification performed in triplicate that had 1 weakly positive signal detected in each run.

patients, and confirmational WB analysis was performed on those with positive results. A subset of these patients demonstrated HTLV-I/II WB seroindeterminate banding patterns, defined by incomplete antibody reactivity to HTLV Env (rgp46-I and rgp46-II) or Gag proteins (figure 1*A*). The WB banding patterns varied among the 12 patients, with 6 demonstrating the commonly observed HTLV-I/II *gag* seroindeterminate status and 6 demonstrating other, less-frequently seen patterns [15, 30]. The mean age of the patients was 41.1 years (4 women and 8 men). Risk factors for HTLV-I exposure included a history of injection drug use (patients 2 and 4), residence in the southern United States (patients 8, 10, and 12), and homosexuality (patient 12). To examine the possibility of prototype HTLV-I infection, we counted the HTLV-I proviral load in PBMCs from these patients, using a sensitive real-time TaqMan quantitative PCR assay [32]. HTLV-I provirus was detected in 5 (42%) of 12 patients (figure 1*B*), which suggests that at least a subset of these individuals could be infected with low levels of prototype HTLV-I.

HTLV-I provirus in HTLV-I/II WB seroindeterminate blood donors. To confirm our hypothesis that HTLV-I/II WB seroindeterminate status may result from infection with low levels

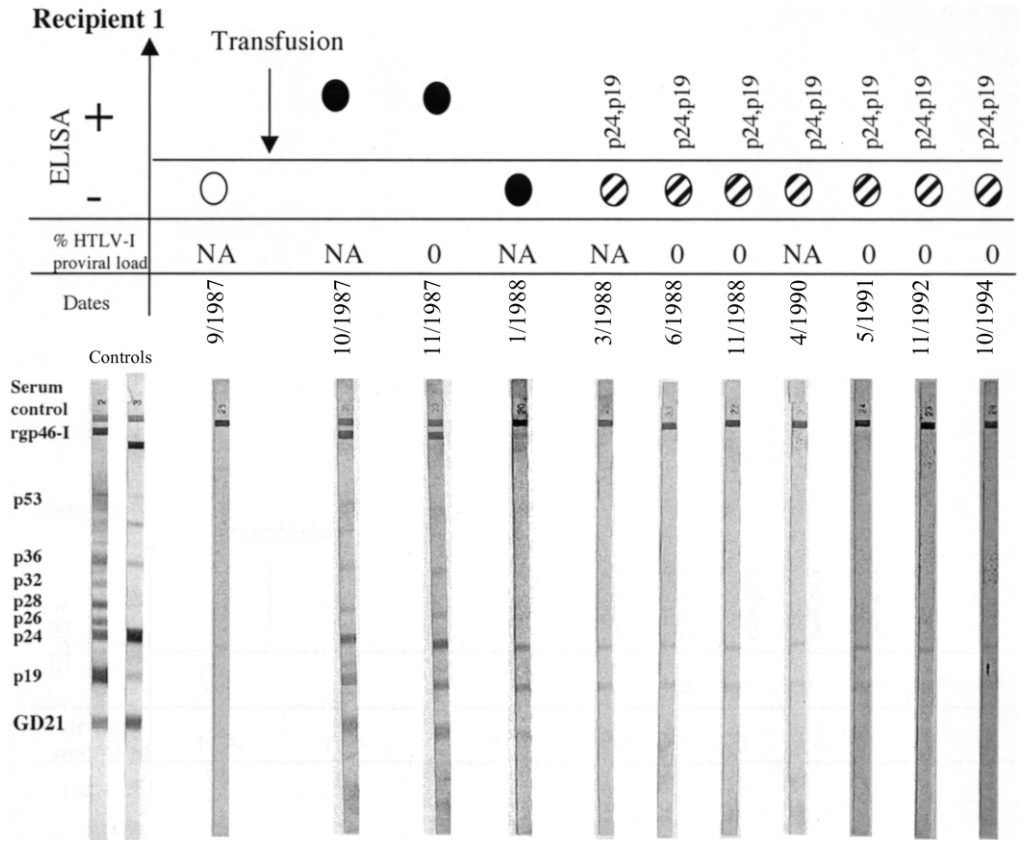

Figure 3. Representative recipient in group 1 with persistent human T lymphotropic virus type I and II (HTLV-I/II) Western blot (WB) seroindeterminate banding patterns. White circles denote HTLV-I/II WB seronegative status; striped circles denote HTLV-I/II WB seroindeterminate status; and black circles denote HTLV-I/II WB positive status. HTLV-I/II ELISA positivity is represented on the *Y*-axis. The HTLV-I proviral load per 10^6 cells for corresponding samples is shown. Evolving WBs performed at 1:100 dilutions are shown underneath.

of prototype HTLV-I, we examined the proviral load in 9 HTLV-I/II ELISA–positive WB seroindeterminate voluntary blood donors from the New York Blood Center (figure 2*A*). All of the blood donors indicated that the United States was their birthplace, and the mean age of this group was 30.1 years (4 women and 5 men). Except for donors NY3 and NY7, no apparent risk factors for HTLV-I infection—such as a history of injection drug use, blood transfusion, or sexual transmission—were noted in this cohort.

As is shown in figure 2*A*, 8 of 9 donors exhibited HTLV-I/II *gag* seroindeterminate status, and 1 individual had weak antibody reactivity to only the p24 antigen. The HTLV-I provirus load, which ranged from 10 to 30 copies/10^6 cells, could be detected in 4 (44.4%) of 9 blood donors (figure 2*B*). These low levels of HTLV-I provirus were similar to those observed in the patients with neurologic symptoms. In addition, these results were validated by another highly sensitive method, HTPCR, using a specific internal probe labeled with P^{32} to the HTLV-I *tax* and *pol* regions [25, 26]. The results of HTPCR correlated with proviral quantitation by use of TaqMan in all cases, except for donors NY2 and NY7 (figure 2*B*), which provides support for a subset of HTLV-I/II WB seroindeterminate individuals being infected with low levels of HTLV-I.

Longitudinal serologic and molecular analysis in transfusion recipients. To investigate whether HTLV-I/II WB seroindeterminate status may represent prior exposure to HTLV-I, we conducted a longitudinal study of a unique cohort of patients from Jamaica who received HTLV-I–positive blood transfusions for various medical reasons. From a total of 66 transfusion recipients, we identified 8 who developed HTLV-I/II WB seroindeterminate status at some point after receiving a trans-

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 4. Group 1: recipients with persistent human T lymphotropic virus type I and II Western blot seroindeterminate banding patterns. The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases*.

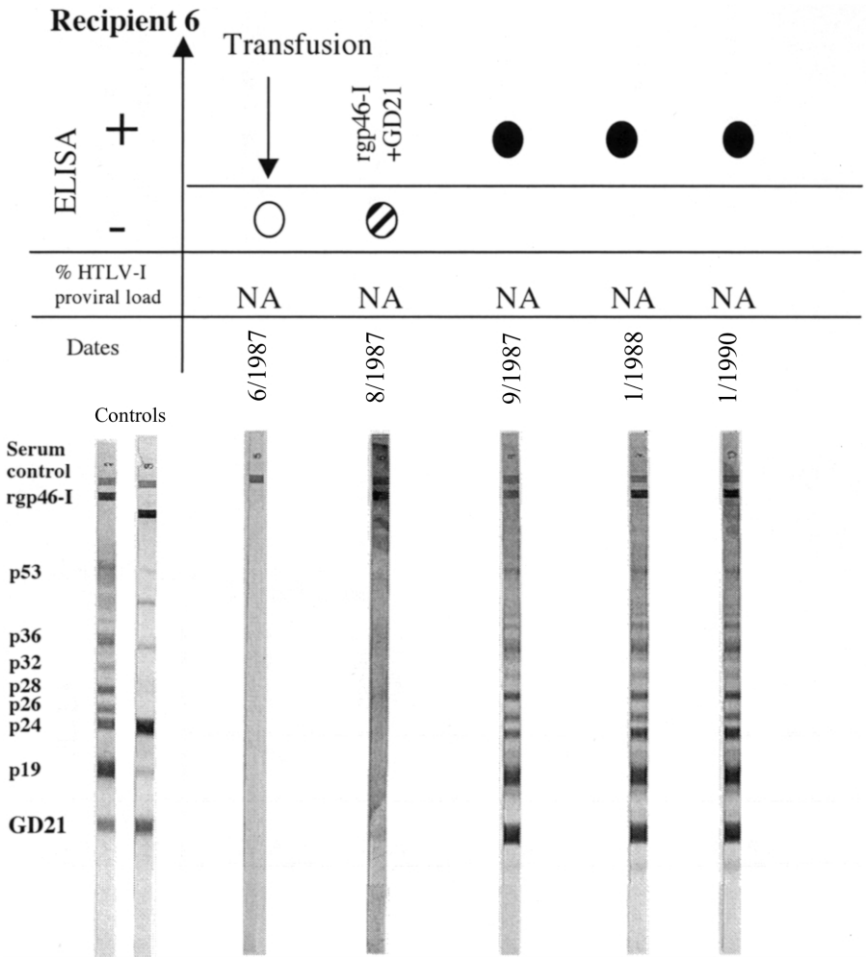

Figure 5. Representative recipient in group 2 who demonstrated seroconversion after blood transfusion. White circles denote human T lymphotropic virus type I and II (HTLV-I/II) Western blot (WB) seronegative status, striped circles denote HTLV-I/II WB seroindeterminate status, and black circles denote HTLV-I/II WB seropositive status. HTLV-I/II ELISA positivity is represented on the *Y*-axis. The HTLV-I proviral load per 10^6 cells of corresponding samples is shown. Evolving WBs performed at 1:100 dilutions are shown underneath.

fusion of HTLV-I–positive blood products (confirmed by ELISA and WB) [2]. HTLV-I/II ELISA and WB were performed on longitudinal samples from these 8 recipients for up to 10 years after transfusion. In addition, corresponding PBMC samples were also evaluated for HTLV-I proviral load.

On the basis of the results of our serologic evaluation, these 8 transfusion recipients were categorized into 3 groups. Group 1 included 4 subjects with sustained HTLV-I/II WB seroindeterminate status (figures 3 and 4); group 2 included 2 subjects who became WB seroindeterminate after transfusion and seroconverted to HTLV-I WB seropositive status (figures 5 and 6); and group 3 included 2 initially WB seronegative subjects who became WB seroindeterminate after transfusion but subsequently reverted to WB seronegative status (figures 7 and 8). Interestingly, transfusion recipients 1, 3, 4, and 6 had ELISA-negative measurements but maintained HTLV-I/II WB seroindeterminate banding patterns according to WB at various points during follow-up. This observation is consistent with the results of a previous report by Soldan et al. [12] in another cohort of HTLV-I/II WB seroindeterminate patients with neurologic symptoms.

In addition to serologic studies, we also evaluated the HTLV-I proviral load in serial PBMC samples. The majority of the patients in group 1 had a nondetectable level of the HTLV-I *pX* gene, except for recipient 2 at 2 months after the transfusion (160 copies/10^6 cells; figure 4). In group 2, low levels of HTLV-I provirus could be detected in recipient 5 corresponding to WB seroindeterminate status. The proviral load in PBMC sam-

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 6. Group 2: seroconversion after transfusion. The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases*.

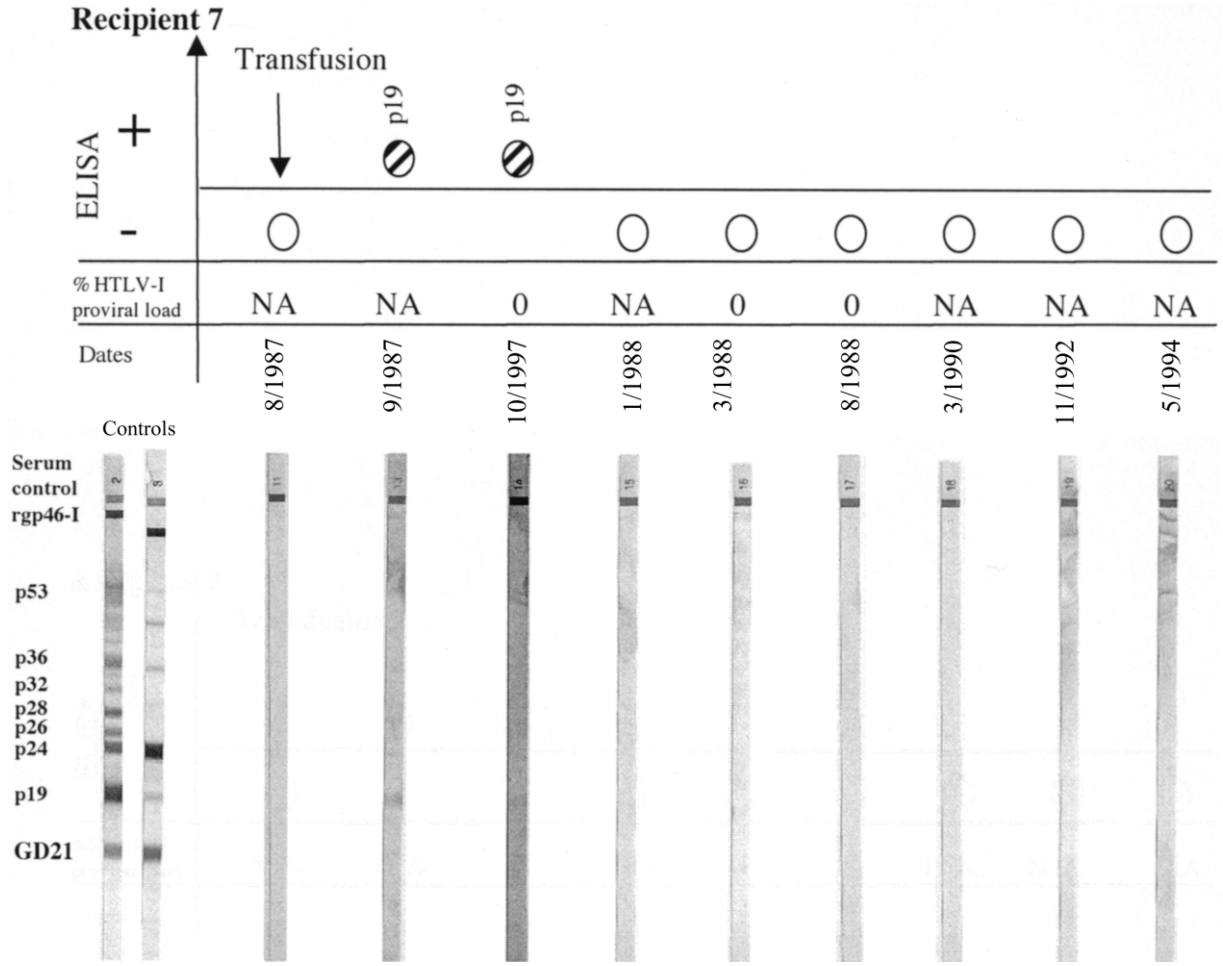

Figure 7. Representative recipient in group 3 who demonstrated seroreversion after transfusion. White circles denote human T lymphotropic virus type I and II (HTLV-I/II) Western blot (WB) seronegative status, striped circles denote HTLV-I/II WB seroindeterminate status, and black circles denote HTLV-I/II WB seropositive status. HTLV-I/II ELISA positivity is represented on the *Y*-axis. The HTLV-I proviral load per 10^6 cells of corresponding samples is shown. Evolving WBs performed at 1:100 dilutions are shown underneath.

ples from this recipient increased from 390 to 4680 copies/10^6 cells after transfusion (figure 6). Patients in group 3 seroreverted, and the HTLV-I provirus load was consistently negative (figures 7 and 8).

HTLV-I Tax11–19–specific CD8$^+$ T cells in HTLV-I/II WB seroindeterminate patients. Patients with HAM/TSP harbor a high percentage of HTLV-I–infected T cells in their PBMCs and exhibit heightened immune responses, whereas asymptomatic carriers tend to have lower percentages of infected cells and fewer HTLV-I–specific T cells [32]. Using an HLA-A*201–restricted HTLV-I Tax11-19 tetramer, we examined HTLV-I–specific T cells in PBMCs from patients with neurologic symptoms with the HLA-A*201 haplotype. Sufficient PBMCs were obtained for tetramer analysis from 2 of 4 WB seroindeterminate patients with the HLA-A*201 haplotype. Low frequencies of HTLV-I specific CD8$^+$ T cells were detected in PBMCs from both patients (0.292% and 0.125% of CD8$^+$ T cells). Figure 9*A* shows a representative tetramer staining profile for an HTLV-I/II WB seroindeterminate patient (patient 10 in figure 1).

To further characterize this population of virus-specific T cells, we purified HTLV-I Tax11-19–specific CD8$^+$ T cells from PBMCs from this WB seroindeterminate individual by positively selecting CD8$^+$ T cells, using MACSbeads (Miltenyi Biotec), followed by FACSsort of the CD8$^+$ and HTLV-I Tax11-19 tetramer–positive population. Subsequent enrichment of this sorted population yielded 9 T cell lines. Analysis by tetramer

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 8. Group 3: seroreversion after transfusion. The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases*.

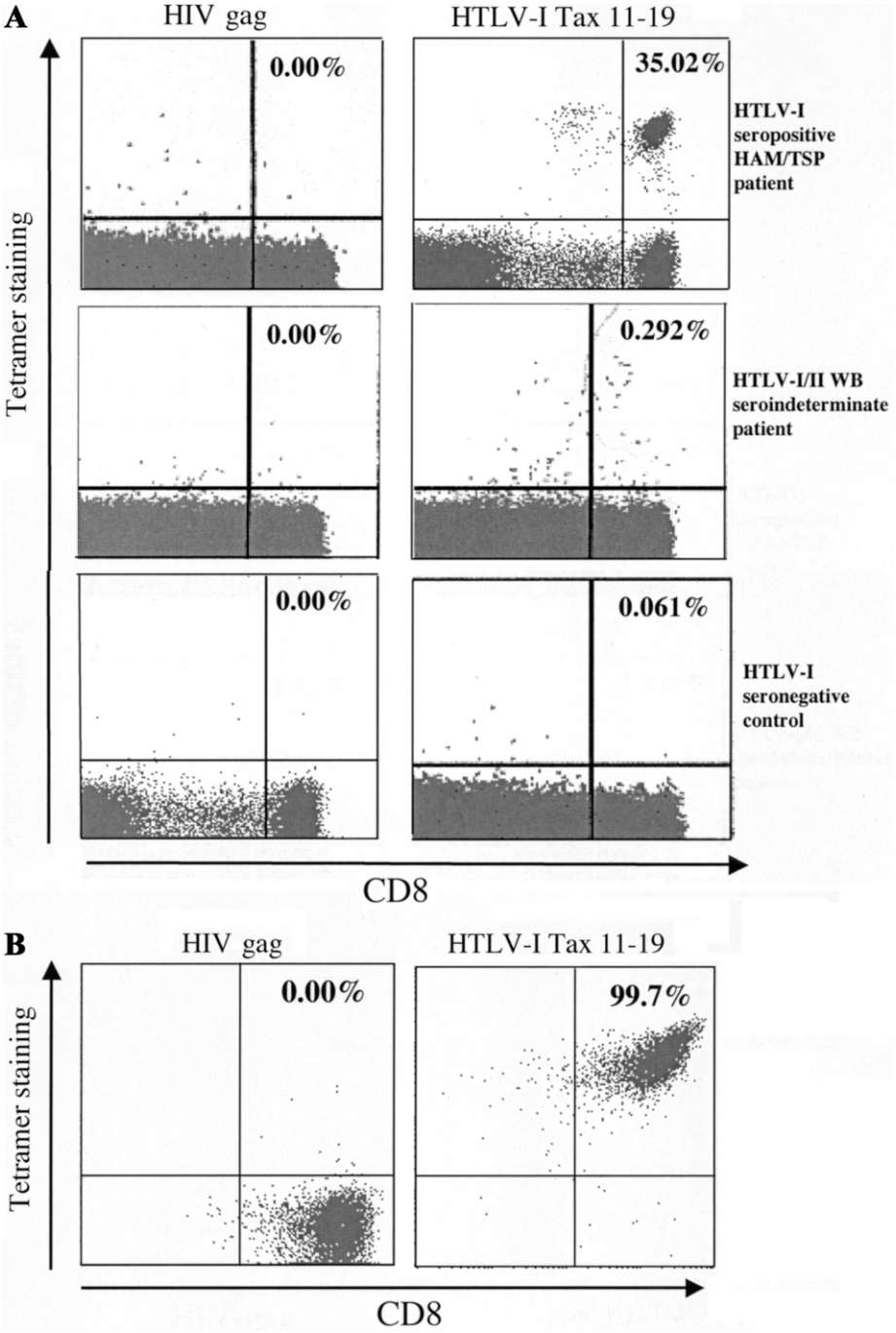

Figure 9. HLA-A*201 human T lymphotropic virus type I (HTLV-I) Tax11-19 tetramer staining. *A,* Staining of peripheral blood mononuclear cells from an HTLV-I/II Western blot (WB) seroindeterminate patient. Tetramer staining profiles of a patient with HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) and a normal donor are shown for comparison. The HLA-A*201 HIV Gag tetramer was used as a negative control. *B,* HTLV-I Tax11-19 tetramer staining of a representative T cell line derived from this patient.

demonstrated that these T cell lines were specific for the HTLV-I Tax11-19 peptide. Figure 9*B* shows a representative tetramer staining profile for these T cell lines.

HTLV-I Tax11-19–specific CD8⁺ cytotoxic T cell activity in HTLV-I/II WB seroindeterminate patients. HTLV-I–specific $CD8^+$ T cells have been suggested to play a central role in the pathogenesis of HAM/TSP. In particular, HTLV-I Tax11-19–specific $CD8^+$ cells were found to represent an extraordinarily high proportion of the total $CD8^+$ population in PBMCs from patients with HAM/TSP. We have demonstrated, in the present study, that low frequencies of HTLV-I Tax11-19–specific $CD8^+$ cells could be detected in PBMCs from WB seroindeterminate

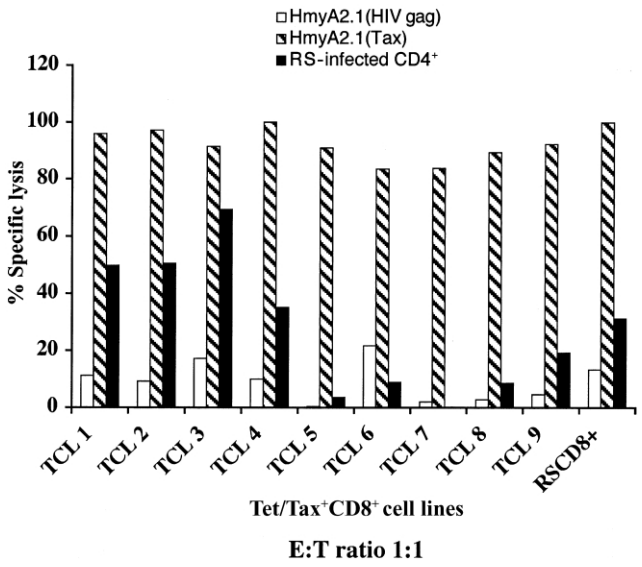

Figure 10. Cytotoxic T lymphocyte activity of T cell lines derived from peripheral blood mononuclear cells from a human T lymphotropic virus type I and II (HTLV-I/II) Western blot (WB) seroindeterminate patient. The target B cell line, HmyA2.1, was pulsed with a 10 μmol/L concentration of either HIV Gag *(white bars)* or HTLV-I Tax11-19 peptide *(striped bars).* T cell lines (TCL 1–9) derived from tetramer-positive $CD8^+$ cells from an HTLV-I/II WB seroindeterminate patient demonstrated specific lysis toward the Tax11-19 peptide. A $CD4^+$ cell line, RS-infected $CD4^+$ derived from a patient with HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) *(black squares),* which expresses an endogenously processed Tax11-19 peptide, was also used to evaluate the cytotoxicity of T cell lines derived from an HTLV-I/II WB seroindeterminate patient. T cell lines 1–4 and 9 demonstrated significant lysis toward RS-infected $CD4^+$ target cells. $RSCD8^+$, a $CD8^+$ cytotoxic T cell line derived from a patient with HAM/TSP, was included as a positive control for T cell cytotoxicity assay. E:T, effector:target.

patients with the HLA-A*201 haplotype and that T cell lines with specificity for the Tax11-19 peptide could be established. It was therefore of interest to evaluate the cytolytic activity of these T cell lines by performing T lymphocyte cytotoxicity assays. CTL clones derived from patients with HAM/TSP have been shown to possess strong cytolytic activity toward antigen-presenting cells pulsed with the Tax11-19 peptide [27, 28, 34–36]. In figure 10, an established CTL clone derived from a patient with HAM/TSP demonstrated strong specific lysis toward Tax11-19 peptide–pulsed HLA-A*201 B cell targets. When we examined cytolytic activity toward Tax11-19 peptide–pulsed target cells of the T cell lines derived from an HTLV-I/II WB seroindeterminate patient with the HLA-A*201 haplotype (patient 10 in figure 1), >80% specific lysis was detected (figure 10).

In addition to lysis of an immunodominant peptide–pulsed target, it was of interest to determine whether the $CD8^+$ HTLV-I Tax11-19 tetramer–positive T cell lines from this HTLV-I/II WB seroindeterminate patient could also target the naturally processed HTLV-I Tax protein. Therefore, we also included in our experiment an HTLV-I–infected $CD4^+$ T cell line, derived from a patient with HAM/TSP who had been shown to express endogenously processed Tax11-19 peptide [16], as target cells for the evaluation of CTL activity. As shown in figure 10, 5 of 9 T cell lines showed specific lysis against this infected $CD4^+$ T cell line, with efficiency comparable to that of the CTL clone derived from the patient with HAM/TSP, which suggests the existence of functionally competent HTLV-I–specific T cells in HTLV-I/II WB seroindeterminate individuals. Collectively, these results support the hypothesis that individuals who are WB seroindeterminate may have had prior exposure to prototypic HTLV-I.

DISCUSSION

The significance of HTLV-I/II WB seroindeterminate status has been controversial. To investigate the implications of this finding, we examined 2 different cohorts of individuals with HTLV-I/II WB seroindeterminate status. In the neurologic and blood donor cohorts, detection of a low HTLV-I proviral load in PBMCs from some individuals (~40%) suggested the possibility of prior exposure to the virus. Such a cross-sectional analysis, however, could not definitively determine a time of exposure. To address this concern, we performed longitudinal studies on prospectively collected samples obtained from a unique cohort of Jamaican transfusion recipients with known exposure to HTLV-I–positive blood products. Of a total of 66 recipients, 8 were identified to have been WB seronegative before transfusion and to subsequently demonstrate HTLV-I/II WB seroindeterminate status. These results demonstrated that exposure to HTLV-I could result in HTLV-I/II WB seroindeterminate status; however, because HTLV infection is endemic in Jamaica, the possibility that the Jamaican cohort may be different from the neurologic and healthy blood donor cohorts cannot be excluded.

Previous studies of HTLV-I/II WB seroindeterminate status have focused on the detection of HTLV-I genes or antibody reactivity by ELISA or WB [11, 13, 15, 16, 20, 31, 33]. In the present study, we extended previous observations and examined T cell responses in HTLV-I/II WB seroindeterminate patients with the HLA-A*201 haplotype. Using an HLA-A*201–restricted HTLV-I Tax11-19–specific tetramer, we detected a low frequency of HTLV-I Tax11-19–specific T cells in PBMCs from WB seroindeterminate patients and found a strong immune response to Tax11-19 by virus-specific T cells. A hallmark immunological characteristic of HTLV-I infection is the spontaneous proliferation of T cells in vitro with no addition of exogenous peptide [29]. We had previously reported [12] that no spontaneous proliferation could be detected in PBMCs from HTLV-I/II WB seroindeterminate individuals; this finding was likely due to the lower frequencies of HTLV-I Tax11-19–specific T cells. Alternatively, because HTLV-I/II WB seroindeterminate status could result from a number of factors [3], it is possible

that the strong immune response against the HTLV-I Tax11-19 peptide that was observed in patient 10 may not be representative of all individuals with HTLV-I/II WB seroindeterminate status.

In serial serum samples collected from individuals with known exposure to HTLV-I by transfusion, the WB seroindeterminate patterns not only appeared transiently before HTLV-I seroconversion or seroreversion but also persisted over a long period of time, without notable changes in banding patterns in some individuals. Such a prolonged WB seroindeterminate status is similar to what has been reported in cases of HIV-1 infection [37]. One might argue that the transient HTLV-I/II WB seroindeterminate banding patterns in these individuals could have been due to the detection of an anti–HTLV-I response in the blood donors rather than in the recipients [1]. However, the persistent HTLV-I/II WB seroindeterminate banding patterns in some transfusion recipients from Jamaica were consistent with patterns previously observed in persons with or without neurologic symptoms [12, 15]; therefore, this could not be explained by cross-reactivity to malaria, because malaria is not endemic in Jamaica. Detection of HTLV-I provirus at low levels in PBMC samples concurrently collected with serum samples strongly argues that exposure to HTLV-I may be associated with HTLV-I/II WB seroindeterminate status in these individuals. Because some transfusion recipients were HTLV-I/II ELISA negative while maintaining the WB seroindeterminate status, we speculate that, if all individuals are to be screened by WB, the prevalence of HTLV-I/II WB seroindeterminate status may be higher than what has been reported.

The possibility that seroindeterminate status may represent low levels of prototype HTLV-I infection has at least 2 public health implications. First, some individuals with seroindeterminate status may be at an increased risk of neurologic symptoms due to either HTLV-I or HTLV-II infection [38–40]; they may thus require heightened monitoring for the clinical onset of disease. Second, our data suggest that screening practices should be enhanced to incorporate WB confirmation or PCR amplification, to eliminate seroindeterminate blood products from circulation. Although screening the entire blood-bank supply by WB would be costly and may not be immediately feasible in many areas around the world, further advancement in HTLV infection–screening techniques that are applicable worldwide will help to establish the clinical significance and true prevalence of HTLV-I/II WB seroindeterminate status. If, indeed, WB seroindeterminate individuals have low levels of current infection, whether they can transmit the infection to others via known routes of transmission, as well as whether they are at increased risk of disease, remains to be determined.

Acknowledgments

We thank Susan Sharrow, for flow-cytometric analysis; and Marcello Morgan, Samantha S. Soldan, and Nahid Ahkyani, for help with human T lymphotropic virus type I and II Western blotting and ELISA.

References

1. Manns A, Hisada M, La Grenade L. Human T-lymphotropic virus type I infection. Lancet **1999**; 353:1951–8.
2. Gessain A, Barin F, Vernant JC, et al. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet **1985**; 2:407–10.
3. Tajima K, Cartier L. Epidemiological features of HTLV-I and adult T cell leukemia. Intervirology **1995**; 38:238–46.
4. Osame M, Izumo S, Igata A, et al. Blood transfusion and HTLV-I associated myelopathy. Lancet **1986**; 2:104–5.
5. Maloney EM, Armien B, Gracia F, et al. Risk factors for human T cell lymphotropic virus type II infection among the Guaymi Indians of Panama. J Infect Dis **1999**; 180:876–9.
6. Leon-Ponte M, Noya O, Bianco N, Echeverria de Perez G. Highly endemic human T-lymphotropic virus type II (HTLV-II) infection in a Venezuelan Guahibo Amerindian group. J Acquir Immune Defic Syndr Hum Retrovirol **1996**; 13:281–6.
7. Briggs NC, Battjes RJ, Cantor KP, et al. Seroprevalence of human T cell lymphotropic virus type II infection, with or without human immunodeficiency virus type 1 coinfection, among US intravenous drug users. J Infect Dis **1995**; 172:51–8.
8. Black FL, Biggar RJ, Lal RB, Gabbai AA, Filho JP. Twenty-five years of HTLV type II follow-up with a possible case of tropical spastic paraparesis in the Kayapo, a Brazilian Indian tribe. AIDS Res Hum Retroviruses **1996**; 12:1623–7.
9. Orland JR, Engstrom J, Fridey J, et al. Prevalence and clinical features of HTLV neurologic disease in the HTLV Outcomes Study. Neurology **2003**; 61:1588–94.
10. Rouet F, Meertens L, Courouble G, et al. Serological, epidemiological, and molecular differences between human T-cell lymphotropic virus type 1 (HTLV-1)-seropositive healthy carriers and persons with HTLV-I Gag indeterminate Western blot patterns from the Caribbean. J Clin Microbiol **2001**; 39:1247–53.
11. Lal RB, Rudolph D, Alpers MP, Sulzer AJ, Shi YP, Lal AA. Immunologic cross-reactivity between structural proteins of human T-cell lymphotropic virus type I and the blood stage of *Plasmodium falciparum*. Clin Diagn Lab Immunol **1994**; 1:5–10.
12. Soldan SS, Graf MD, Waziri A, et al. HTLV-I/II seroindeterminate Western blot reactivity in a cohort of patients with neurological disease. J Infect Dis **1999**; 180:685–94.
13. Garin B, Gosselin S, de The G, Gessain A. HTLV-I/II infection in a high viral endemic area of Zaire, Central Africa: comparative evaluation of serology, PCR, and significance of indeterminate Western blot pattern. J Med Virol **1994**; 44:104–9.
14. Caterino-de-Araujo A, de los Santos-Fortuna E, Meleiro MC, et al. Sensitivity of two enzyme-linked immunosorbent assay tests in relation to western blot in detecting human T-cell lymphotropic virus types I and II infection among HIV-1 infected patients from Sao Paulo, Brazil. Diagn Microbiol Infect Dis **1998**; 30:173–82.
15. Cesaire R, Bera O, Maier H, et al. Seroindeterminate patterns and seroconversions to human T-lymphotropic virus type I positivity in blood donors from Martinique, French West Indies. Transfusion **1999**; 39:1145–9.
16. Cohen CJ, Sarig O, Yamano Y, Tomaru U, Jacobson S, Reiter Y. Direct phenotypic analysis of human MHC class I antigen presentation: visualization, quantitation, and in situ detection of human viral epitopes using peptide-specific, MHC-restricted human recombinant antibodies. J Immunol **2003**; 170:4349–61.

17. Segurado AA, Malaque CM, Sumita LM, Pannuti CS, Lal RB. Laboratory characterization of human T cell lymphotropic virus types 1 (HTLV-1) and 2 (HTLV-2) infections in blood donors from Sao Paulo, Brazil. Am J Trop Med Hyg **1997**; 57:142–8.
18. Mangano AM, Remesar M, del Pozo A, Sen L. Human T lymphotropic virus types I and II proviral sequences in Argentinian blood donors with indeterminate Western blot patterns. J Med Virol **2004**; 74:323–7.
19. Waziri A, Soldan SS, Graf MD, Nagle J, Jacobson S. Characterization and sequencing of prototypic human T-lymphotropic virus type 1 (HTLV-1) from an HTLV-1/2 seroindeterminate patient. J Virol **2000**; 74:2178–85.
20. Mahieux R, Horal P, Mauclere P, et al. Human T-cell lymphotropic virus type 1 gag indeterminate western blot patterns in Central Africa: relationship to *Plasmodium falciparum* infection. J Clin Microbiol **2000**; 38:4049–57.
21. Morozov VA, Ellerbrok H, Fleischer C, Brackmann HH, Pauli G. Defective human T-cell leukaemia virus type 1 (HTLV-1) genomes: no evidence in serologically indeterminate German blood donors but new type detected in established cell lines. J Med Virol **2002**; 66:102–6.
22. Ramirez E, Cartier L, Rios M, Fernandez J. Defective human T-cell lymphotropic virus type I (HTLV-I) provirus in 10 Chilean seronegative patients with tropical spastic paraparesis or HTLV-I-associated myelopathy. J Clin Microbiol **1998**; 36:1811–3.
23. Ramirez E, Fernandez J, Cartier L, Villota C, Rios M. Defective human T-cell lymphotropic virus type I (HTLV-I) provirus in seronegative tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) patients. Virus Res **2003**; 91:231–9.
24. Manns A, Wilks RJ, Murphy EL, et al. A prospective study of transmission by transfusion of HTLV-I and risk factors associated with seroconversion. Int J Cancer **1992**; 51:886–91.
25. Rios M, Khabbaz RF, Kaplan JE, Hall WW, Kessler D, Bianco C. Transmission of human T cell lymphotropic virus (HTLV) type II by transfusion of HTLV-I–screened blood products. J Infect Dis **1994**; 170:206–10.
26. Puccioni-Sohler M, Rios M, Bianco C, et al. An inverse correlation of HTLV-I viral load in CSF and intrathecal synthesis of HTLV-I antibodies in TSP/HAM. Neurology **1999**; 53:1335–9.
27. Kubota R, Nagai M, Kawanishi T, Osame M, Jacobson S. Increased HTLV type 1 tax specific CD8+ cells in HTLV type 1-associated myelopathy/tropical spastic paraparesis: correlation with HTLV type 1 proviral load. AIDS Res Hum Retroviruses **2000**; 16:1705–9.
28. Kubota R, Soldan SS, Martin R, Jacobson S. Selected cytotoxic T lymphocytes with high specificity for HTLV-I in cerebrospinal fluid from a HAM/TSP patient. J Neurovirol **2002**; 8:53–7.
29. Jacobson S, McFarlin DE, Robinson S, et al. HTLV-I-specific cytotoxic T lymphocytes in the cerebrospinal fluid of patients with HTLV-I-associated neurological disease. Ann Neurol **1992**; 32:651–7.
30. Lal RB, Rudolph DL, Coligan JE, Brodine SK, Roberts CR. Failure to detect evidence of human T-lymphotropic virus (HTLV) type I and type II in blood donors with isolated gag antibodies to HTLV-I/II. Blood **1992**; 80:544–50.
31. Lal RB, Brodine S, Kazura J, Mbidde-Katonga E, Yanagihara R, Roberts C. Sensitivity and specificity of a recombinant transmembrane glycoprotein (rgp21)-spiked Western immunoblot for serological confirmation of human T-cell lymphotropic virus type I and type II infections. J Clin Microbiol **1992**; 30:296–9.
32. Yamano Y, Nagai M, Brennan M, et al. Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific $CD8^+$ T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). Blood **2002**; 99:88–94.
33. Khabbaz RF, Heneine W, Grindon A, Hartley TM, Shulman G, Kaplan J. Indeterminate HTLV serologic results in U.S. blood donors: are they due to HTLV-I or HTLV-II? J Acquir Immune Defic Syndr **1992**; 5: 400–4.
34. Nagai M, Kubota R, Greten TF, Schneck JP, Leist TP, Jacobson S. Increased activated human T cell lymphotropic virus type I (HTLV-I) Tax11-19–specific memory and effector $CD8^+$ cells in patients with HTLV-I–associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. J Infect Dis **2001**; 183:197–205.
35. Ureta-Vidal A, Pique C, Garcia Z, et al. Human T cell leukemia virus type I (HTLV-I) infection induces greater expansions of CD8 T lymphocytes in persons with HTLV-I–associated myelopathy/tropical spastic paraparesis than in asymptomatic carriers. J Infect Dis **2001**; 183: 857–64.
36. Biddison WE, Kubota R, Kawanishi T, et al. Human T cell leukemia virus type I (HTLV-I)-specific CD8+ CTL clones from patients with HTLV-I-associated neurologic disease secrete proinflammatory cytokines, chemokines, and matrix metalloproteinase. J Immunol **1997**; 159:2018–25.
37. Rhodes D, Solomon A, Bolton W, et al. Identification of a new recipient in the Sydney Blood Bank Cohort: a long-term HIV type 1-infected seroindeterminate individual. AIDS Res Hum Retroviruses **1999**; 15: 1433–9.
38. Jacobson S, Lehky T, Nishimura M, Robinson S, McFarlin DE, Dhib-Jalbut S. Isolation of HTLV-II from a patient with chronic, progressive neurological disease clinically indistinguishable from HTLV-I-associated myelopathy/tropical spastic paraparesis. Ann Neurol **1993**; 33:392–6.
39. Murphy EL, Glynn SA, Fridey J, et al. Increased incidence of infectious diseases during prospective follow-up of human T-lymphotropic virus type II- and I-infected blood donors. Retrovirus Epidemiology Donor Study. Arch Intern Med **1999**; 159:1485–91.
40. Biglione MM, Pizarro M, Salomón HE, Berría MI. A possible case of myelopathy/tropical spastic paraparesis in an Argentinian woman with human T lymphocyte virus type II. Clin Infect Dis **2003**; 37:456–8.